

From the Department of Pharmacology, University of Lund, Sweden

**STUDIES ON THE EXCITATION - CONTRACTION COUPLING IN
FROG SKELETAL MUSCLE**

By

K. - E. ANDERSSON
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Lund 1973
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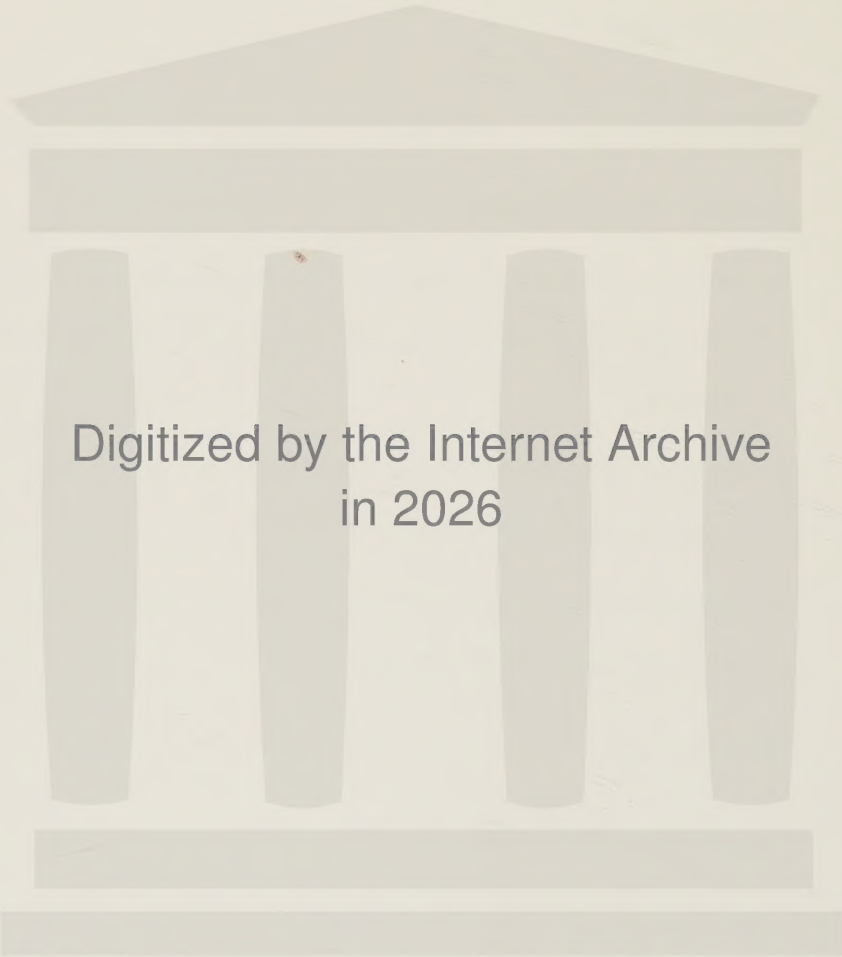
The following account summarizes the five papers

- I ANDERSSON, K.-E. Effects of chlorpromazine, imipramine, and quinidine on action potential and tension development in single skeletal muscle fibres of the frog.
Acta physiol. scand. 1973. In press.
- II ANDERSSON, K.-E. Effects of chlorpromazine, imipramine, and quinidine on the mechanical activity of single skeletal muscle fibres of the frog. Acta physiol. scand. 1972. 85. 532-546.
- III ANDERSSON, K.-E. and K.A.P. EDMAN, Effects of lanthanum on the coupling between membrane excitation and contraction of isolated frog muscle fibres. Acta physiol. scand. 1973. In press.
- IV ANDERSSON, K.-E. and K.A.P. EDMAN, Effects of lanthanum on potassium contractures of isolated twitch muscle fibres of the frog. Acta physiol. scand. 1973. In press.
- V ANDERSSON, K.-E. The effect of hypertonicity on the time course of the active state in single skeletal muscle fibres of the frog. Acta physiol. scand. 1973. In press.

In the text the papers are referred to by their Roman numerals.

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1. INTRODUCTION

The present study concerns the effects of certain drugs, lanthanum, calcium, and hypertonicity of the extracellular medium on membrane potential and contractile responses of isolated skeletal muscle fibres from the frog. Its main object was to obtain further information about the different steps in the excitation-contraction coupling, i.e. the sequence of events linking membrane excitation to activation of the contractile machinery in the muscle.

Depolarization of the cell membrane is the first step in the excitation-contraction coupling and in twitch muscle fibres, this process is normally initiated by the action potential. Experimentally a graded depolarization of the fibre can be achieved by increasing the extracellular potassium concentration. Membrane depolarization causes an increase in the myoplasmic calcium concentration, attributable to a release of calcium from the terminal sacs of the sarcoplasmic reticulum. However, little is known about the mechanism of the release. At a sufficient concentration of free calcium in the myofibrillar space, the contractile system is activated and the fibre contracts (see reviews by Sandow 1965, 1970, Ebashi and Endo 1968, Zachar 1971).

Relaxation of the muscle fibre occurs when activator calcium is removed from the contractile machinery. This is achieved by an active transport mechanism located in the membranes of the sarcoplasmic reticulum (Sandow 1965, 1970, Ebashi and Endo 1968).

The kinetics of release and re-uptake of activator calcium can be regarded as important determinants of tension development

and relaxation in a muscle fibre. Agents known to influence these processes may therefore be used as tools for investigating the different steps in the excitation-contraction coupling in muscle. It has been shown that drugs such as chlorpromazine, imipramine, and quinidine inhibit the sequestration of calcium by isolated sarcoplasmic reticulum (Balzer, Makinose and Hasselbach 1968, Fuchs, Gertz and Briggs 1968). Such an effect, if it occurs in the living fibre, would be expected to slow the elimination of calcium from the myofibrillar space and, therefore, to influence the duration of the contraction and the rate of relaxation. In addition, these drugs are known to have a 'stabilizing' effect on the cell membrane (Shanes 1958 a, b) and might be expected to influence the excitation of the muscle. When the present study began, little information was available concerning the effects of chlorpromazine and imipramine on excitation and contraction of skeletal muscle. The actions of quinidine and its optical isomer quinine had been investigated previously on whole muscle function (Santesson 1897, Harvey 1939, Krayner and George 1951, Lammers and Ritchie 1955, Isaacson and Sandow 1967). The use of the single fibre preparation in the present investigation, enabled a detailed study of the effects of chlorpromazine, imipramine, and quinidine on both the electrical and mechanical activities in the same cell and made it possible to correlate the two events. The results of these studies are presented in papers I and II.

A twitch muscle fibre contracts in response to a high external potassium concentration, but relaxes again despite sustained depolarization. The relationship between peak contracture tension and log extracellular potassium concentration (or membrane potential) provides a steep S-shaped curve (Hodgkin and Horowicz 1960).

Contractile activity is initiated at membrane potential levels close to -50 mV (mechanical threshold). The relationship between contracture tension and membrane potential, and also the time course of the potassium contracture is influenced by the extracellular calcium concentration (Lüttgau 1963, Pauschinger, Lorkovic and Brecht 1964, Milligan 1965, Caputo and Gimenez 1967, Foulks and Perry 1967, Frankenhaeuser and Lännergren 1967, Etzensperger 1970 a). Extracellular calcium may produce these effects by acting on the mechanism that governs the release of calcium from the sarcoplasmic reticulum and/or by affecting the process of re-uptake of calcium by this structure. The trivalent metal ion lanthanum seemed to offer a means to distinguish these two possibilities, as this ion has been demonstrated to have effects on various membrane functions very similar to those produced by high concentrations of calcium (Takata et al. 1966). Lanthanum binds much more strongly to the membrane than calcium (Hagiwara and Takahashi 1967), and evidence from electron microscopical studies and from investigations on the effects of lanthanum on transmembrane calcium fluxes support the view that lanthanum does not pass the cell membrane to any significant degree (Lesseps 1967, Weiss 1970, Langer and Frank 1972, van Breemen et al. 1972). Papers III and IV describe experiments carried out to investigate the effects of lanthanum on twitch and tetanus responses and on potassium-induced contractures of single muscle fibres.

Hypertonicity of the extracellular medium is known to reduce the tension output of frog skeletal muscle (Overton 1902, Ernst 1926, Howarth 1958, Caputo 1968, Edman and Andersson 1968, Gordon and Godt 1970). The decrease in contractile force has mainly been attributed to a direct effect on the contractile proteins caused

by an increased intracellular ionic strength (Edman and Andersson 1968, Gordon and Godt 1970). However, the reduction of the isometric twitch response is greater than the reduction of the tetanus tension (Gordon and Godt 1970), suggesting that the osmotic change in a more specific way affects the kinetics of activation of the contractile machinery. The aim of the experiments described in paper V was to investigate in more detail the mechanics of the contractile changes produced by hypertonicity.

As to its functional behaviour, the contractile machinery of skeletal muscle may be represented by an active, contractile unit coupled in series with a passive, elastic element (Hill 1938). Due to this arrangement the mechanical output of a muscle fibre in a single, isometric twitch does not give a true picture of the actual capacity of the contractile unit to produce tension during contraction. A certain time is required to stretch the series elastic element, and this will cause a substantial delay in the external manifestation of the activity. The capacity of the contractile unit to produce tension, defined as the active state, can be determined by arranging the recordings in such a way that no movement occurs in the contractile system. Different approaches may be used for this purpose (Ritchie 1954 a, b, Macpherson and Wilkie 1954, Edman 1970, Edman and Kiessling 1971).

2. METHODS

Preparation and mounting. Single muscle fibres, dissected from the ventral head of the semitendinosus muscle of Rana temporaria were used. In some experiments complementary measurements of resting and action membrane potentials were made on small bundles of muscle fibres (containing 3-8 fibres). The fibres were mounted horizontally in a jacketed, temperature-controlled Lucite chamber. They were suspended between an adjustable lever and an RCA 5734 mechano-electric transducer. The length of the fibre and the degree of shortening could be controlled by means of two adjustable screws in front of and behind the lever. Sarcomere length was determined by direct microscopy using the approach described by Edman (1966). The chamber contained 1.2 ml fluid and allowed rapid exchanges of solution. Further details of the apparatus and the arrangements used for production of potassium contractures and for determination of the active state are described in papers I-V.

Stimulation. The fibres were made to contract either by electrical stimulation via a multi-electrode assembly or by flushing a solution containing a high potassium concentration through the chamber.

Recording. Tension was recorded by means of an RCA 5734 mechano-electric transducer. The signal from the transducer was amplified and displayed on a Tektronix 502 A oscilloscope and/or on paper by means of an Elema-Schönander ink-jet recorder. In some experiments a potentiometer recorder was used.

Membrane potentials were recorded intracellularly by means of conventional Ling-Gerard glass capillary electrodes. The reference electrode was an Ag-AgCl electrode connected to the bath through an Agar bridge. The signals were fed into an electrometer of high input impedance and displayed on a Tektronix 502 A oscilloscope. The rate of rise and rate of fall of the action potential was recorded by electrical differentiation using an RC-circuit with a time constant of 0.1 ms.

Solutions. The compositions of the various solutions used are described in papers I-V.

3. RESULTS AND DISCUSSION

A. Effects of chlorpromazine, imipramine, and quinidine on electrical and mechanical activities of frog muscle fibres

I. Paper I describes the effects of chlorpromazine, imipramine, and quinidine on resting and action membrane potentials of single muscle fibres and also the effects of these drugs on twitch and tetanus responses and on the time course of the active state. In the concentrations used (chlorpromazine 10^{-6} - 3×10^{-5} M, imipramine and quinidine 10^{-5} - 10^{-4} M) neither of the drugs exerted any effect on the resting membrane potential of the fibres. They all caused similar graded changes of the action potential: a decrease in the rate of rise, rate of fall, and overshoot. They also increased the duration of the action potential, measured at the -50 mV level. At high concentrations the drugs blocked the electrical activity. These changes are common to agents having a membrane stabilizing action (Shanes 1958 a, b) such as calcium (Ishiko and Sato 1957, Etzensperger 1970 a), lanthanum (III), and local anaesthetics (Karzel, Draper and Freibel 1965, Etzensperger 1970 b).

Chlorpromazine, imipramine, and quinidine all potentiated the isometric twitch and prolonged the time to peak twitch tension and the total duration of the twitch. These changes could be attributed to an increased duration of the active state. Tetanic tension was not reduced. When high concentrations of the drugs were used (chlorpromazine $\geq 3 \times 10^{-5}$ M, imipramine and quinidine $> 5 \times 10^{-5}$ M), the twitch amplitude diminished and the mechanical response eventually disappeared. This was demonstrated to be due to local blocks of excitation causing incomplete activation of the

fibre. Such an effect is likely to be the cause of the reduced twitch response observed in whole muscle following treatment with chlorpromazine and quinidine (Kopera and Armitage 1954, Isaacson and Sandow 1967, Balzer and Hellenbrecht 1969).

The effects on the action potential produced by chlorpromazine, imipramine, and quinidine may to a great extent account for their twitch potentiating action. As demonstrated previously (Sandow and Preiser 1964, Sandow, Taylor and Preiser 1965, Edman, Grieve and Nilsson 1966, Taylor, Preiser and Sandow 1972), there appears to be a quantitative relation between the duration of the action potential and the duration of the active state, which has been interpreted to mean that the action potential governs the time during which activator calcium is released into the myofibrillar space. However, other factors may also be of importance for the twitch potentiating effect of these drugs. As is shown in paper II, they shift the curve relating peak contracture tension to membrane potential to more negative potentials. Furthermore, they inhibit the re-uptake of calcium by the sarcoplasmic reticulum (Balzer, Makinose and Hasselbach 1968, Balzer 1972). All these factors may contribute to an increase in the duration of the active state and a potentiation of the twitch.

II. The first part of paper II deals with the effects of chlorpromazine, imipramine, and quinidine on potassium-induced contractures. It was found that the drugs did not affect the amplitude of the potassium contracture, but markedly prolonged the plateau and reduced the rate of relaxation. They also shifted the curve relating peak contracture tension to external potassium concentration to lower potassium concentrations. This was somewhat

unexpected, as membrane stabilizers such as calcium (Lüttgau 1963, Frankenhaeuser and Lännergren 1967, Etzensperger 1970 a), lanthanum (IV), and conventional local anaesthetics e.g. tetracaine and procaine (Lüttgau and Oetliker 1968, Etzensperger 1970 b), which are believed to exert their effects mainly on the cell membrane, generally shift the curve to higher potassium concentrations (corresponding to less negative membrane potentials). However, the obtained results are explainable if chlorpromazine, imipramine, and quinidine act on the intracellular calcium store as well, thereby increasing the effectiveness of a given depolarization to raise the calcium concentration in the myoplasm. The possibility exists that these drugs, by making the sarcoplasmic reticulum less effective to accumulate calcium, increase the resting concentration of calcium in the myoplasm. The calcium released by depolarization, when added to the increased resting calcium content in the myoplasm would cause an increased amount of activator available for the contractile system. In support of such a view it has been shown in recent experiments on frog muscle in this laboratory (Batra 1973) that chlorpromazine, imipramine, and quinidine can release some of the calcium that has previously been taken up by isolated sarcoplasmic reticulum (see also Carvalho 1968, Bondani and Karler 1970, Balzer 1972).

The prolongation of the plateau phase of the contracture and the decreased rate of relaxation caused by these drugs are very similar to the effects produced by lanthanum (IV). These effects may be explained by assuming that the drugs due to their inhibiting effect on the uptake of calcium in the sarcoplasmic reticulum (Balzer, Makinose and Hasselbach 1968), slow the elimination of activator from the myofibrillar space. Another possibility

is that the drugs prolong the release of activator calcium by slowing the inactivation of the release mechanism (cf discussion in IV).

Further support for the view that chlorpromazine, imipramine, and quinidine have actions on intracellular calcium storing structures are given in the second part of paper II. It is known that the drugs investigated in high concentrations produce slowly developing contractures in skeletal muscle (Isaacson and Sandow 1967, Balzer and Hellenbrecht 1969, Huddart 1971 a, b). The mechanism of the contractures has been explained as resulting from an increased influx of calcium from the extracellular medium in combination with a reduced uptake of calcium by the sarcoplasmic reticulum (Balzer and Hellenbrecht 1969). The results presented in paper II provide evidence against this view in that contractures could be evoked in a medium free of calcium. Furthermore, the contractures seem to be independent of changes in membrane potential. These findings favour the idea of a direct action of the drugs on an intracellular calcium store, leading to calcium release and contracture development.

B. Effects of lanthanum on electrical and mechanical activities of frog muscle fibres.

III. The experiments described in paper III were aimed at elucidating the actions of lanthanum on twitch and tetanus responses of isolated muscle fibres. It was demonstrated that lanthanum increased the latent period, increased the rate of tension development, potentiated the peak twitch amplitude, and prolonged the relaxation period. These effects could largely be attributed to simultaneous changes of the action potential and the mechanical threshold. Lanthanum decreased the amplitude and reduced the rate of rise and rate of fall of the action potential, and increased the action potential duration. As demonstrated in paper IV, lanthanum also increased the mechanical threshold.

In fibres that had depolarized from approximately -90 mV to -45 mV, lanthanum was able to restore the membrane potential to its original value. Furthermore, in the presence of 0.1 mM lanthanum the fibre retained a normal resting membrane potential and twitch response for at least 4-5 h in a calcium-free medium.

These experiments suggest that lanthanum and calcium act on a common site in the membrane, and that lanthanum can substitute for calcium in the membrane function.

IV. There is still incomplete information as to the mechanism by which calcium is released from the intracellular store (see e.g. Zachar 1971). The actions of calcium on the time course of the potassium contracture (Lüttgau 1963, Frankenhaeuser and Lännergren 1967) may be interpreted to mean that the release of activator calcium in some way is controlled by the extracellular calcium, probably by an action on a membrane site. The experiments

described in paper IV were performed as an attempt to further elucidate the mechanism in the membrane that governs the release of activator calcium. Lanthanum was used, as this metal ion has been shown to replace calcium in the membrane function (III) and probably does not penetrate the cell membrane to any significant degree (Lesseps 1967, Langer and Frank 1972).

The effects of lanthanum on potassium-induced contractures were investigated. It was found that lanthanum (0.01-1.0 mM) exerted two distinct effects: 1. it reduced the rate of tension development and decreased the peak contracture amplitude; 2. it prolonged the contracture plateau and decreased the rate of relaxation during sustained depolarization. The effects under point 1. (obtained with 0.5-1.0 mM lanthanum) can be explained by assuming that lanthanum reduces the rate at which activator calcium is released at any given depolarization. Thus, the effectiveness of a depolarization step to release calcium seemed to be decreased. Evidence in support of this view was the finding that lanthanum caused a shift of the curve relating peak contracture tension to membrane potential to less negative potentials.

It is of interest to compare the lanthanum effects on the contracture with those produced by chlorpromazine, imipramine, and quinidine. The drugs apparently increase the effectiveness of a given depolarization to release calcium as the curve relating peak contracture tension to membrane potential is shifted to more negative potentials (II). Both lanthanum and the drugs produce the same type of changes of the action potential, suggesting that they have a common stabilizing action on the membrane. Assuming that the actions of lanthanum are exerted on the cell membrane exclusively, and that the drugs act both on the membrane and on

the internal store of calcium, these findings may be taken as evidence that the membrane stabilizing effects of the drugs are of less importance for tension development than their actions on the intracellular structures.

The prolongation of the contracture plateau and decreased rate of relaxation produced by lanthanum in low concentrations (≥ 0.01 mM) may be interpreted to mean that lanthanum by actions on a membrane site reduces the rate of inactivation of the calcium releasing mechanism (for discussion, see IV).

C. Effects of hypertonicity on electrical and mechanical activities of frog muscle fibres.

V. There are reasons to believe that the active state reflects the concentration of calcium that is bound at a given moment to the contractile system of a muscle fibre (Edman, Grieve and Nilsson 1966, Ebashi and Endo 1968, Edman 1970, Sandow 1970, Edman and Kiessling 1971). The active state curve may therefore provide useful information for the evaluation of the kinetics of activator calcium at the contractile sites in the cell. In paper V the effects of hypertonicity of the extracellular medium on the tension development of single muscle fibres were studied, and special attention was given to effects on the time course of the active state. Active state was determined by three different methods providing data on the rising phase, the duration of the plateau, and the rate of decay of the active state. Some interesting differences were found between the responses to a single stimulus and to repetitive stimulation. During a single, isometric twitch the plateau phase of the active state was abbreviated in hyper-

tonic medium. During repetitive stimulation, on the other hand, there was a progressive increase in the duration of each respective active state cycle, and a slowing of the rate of decay of the activity. It could be excluded that changes of the action potential during repetitive stimulation were the cause of this difference. The apparent discrepancy in results observed in a single twitch and in response to repetitive stimulation may be explained in the following way: There is evidence that hypertonicity reduces the amount of calcium released by electrical stimulation (Homsher and Briggs 1968, Ashley and Ridgway 1970). This will lead to an abbreviation (and possibly to a reduced intensity) of the active state in response to a single stimulus. As hypertonicity reduces the rate of resequestration of calcium by the sarcoplasmic reticulum (Martonosi and Feretos 1964, de Meis 1968), repetitive stimulation may lead to an accumulation of activator calcium in the myofibrillar space. This, in turn, will cause the active state to reach maximal intensity. Furthermore, there will be an increase in the duration of the active state and a slowing of the decay of activity.

4. SUMMARY

The aim of the present study was to obtain information about different steps in the excitation-contraction coupling in frog skeletal muscle. For this purpose, the drugs chlorpromazine, imipramine, and quinidine, the metal ions lanthanum and calcium, and hypertonicity of the extracellular medium were used as investigational tools. The obtained results can be summarized as follows:

A. Chlorpromazine, imipramine, and quinidine did not affect the resting membrane potential of the fibres but produced changes of the action potential probably attributable to a non-specific membrane stabilizing effect. There was a concentration dependent potentiation of the isometric twitch that could be largely explained by changes of the action potential. No effect on tetanic tension was observed. In high concentrations, the drugs blocked the mechanical response to electrical stimulation. The drugs did not reduce the peak amplitude of potassium-induced contractures, but prolonged the contracture plateau and decreased the rate of relaxation. Furthermore, they shifted the curve relating peak contracture tension to membrane potential to more negative potentials. It is concluded that the drugs have actions on the release and re-uptake of calcium inside the cell not immediately related to their membrane stabilizing effect.

B. Lanthanum had no effect on the resting membrane potential of the fibres, but produced changes of the action potential similar to those evoked by chlorpromazine, imipramine, and quinidine. The metal ion had characteristic effects on the isometric twitch that were well explainable by simultaneous changes of the action potential and mechanical threshold. Lanthanum prevented membrane

depolarization from occurring in a calcium-free medium and preserved the twitch response and potassium contracture for many hours in the absence of extracellular calcium. In low concentrations lanthanum prolonged the contracture plateau and markedly decreased the rate of relaxation of the potassium contracture. In relatively high concentrations, lanthanum also reduced the rate of tension development and the amplitude of the contracture. The curve relating peak contracture tension to membrane potential was shifted to less negative potentials. It is concluded that lanthanum can replace calcium in the membrane function, and that the metal ion by actions on mechanisms in the membrane normally governed by calcium, produces its effects on membrane potential and contractile responses of skeletal muscle.

C. Hypertonicity of the extracellular medium had insignificant effects on resting and action membrane potentials of skeletal muscle fibres. During a single isometric twitch the active state was abbreviated, probably due to a diminished release of calcium from the sarcoplasmic reticulum. During repetitive stimulation, on the other hand, the duration of the active state was prolonged. This can be explained by an accumulation of calcium in the myofibrillar space due to a reduced rate of re-uptake of the calcium ion by the sarcoplasmic reticulum.

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